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绿色荧光蛋白基因作为报告基因在水稻基因转化中的应用研究*

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摘要：本研究中，构建了含有编码绿色荧光蛋白的改进型基因质粒 pJPM5。用基因枪法分别把 pJPM5 和另一带有绿色荧光蛋白基因的质粒 pSBG700 转入水稻 TNG67 愈伤组织。用 Southern 杂交法证实了转基因的存在，而且表明多数转基因植株含有 1 到 8 个拷贝的转基因。取 2 个月的转基因植株上的叶片用于分析绿色荧光蛋白基因表达。用 SLM－8000 荧光分析仪定量测定绿色荧光蛋白。多数转基因植株具有很高的绿色荧光蛋白信号。虽然水稻植株有少量自发荧光，但是绿色荧光蛋白基因表达出的绿色荧光蛋白信号比植株的自发荧光强得多，其测定不会受自发荧光的太大影响。在荧光显微镜下观察到了绿色荧光蛋白基因的表达。借助观察分析绿色荧光蛋白基因的瞬时表达，本研究还发现基因枪法转化中，如果两枪的气压为 900psi & 1350 psi，比两枪的气压都为 900psi 或者 1350psi 更好，因其能使质粒进入更多的细胞。研究结果表明，绿色荧光蛋白基因可以作为水稻（甚至小麦、玉米）转基因研究中的报告基因。研究还显示，MAR 序列能明显增强绿色荧光蛋白基因的表达能力（这一结果在另文讨论）.

关键词：绿色荧光蛋白；报告基因；遗传转；水稻

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The Applications of *GFP* as a Reporter Gene in Rice Genetic Transformation*

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Abstract：In this study，a plasmid pJPM5 containing modified GFP gene which encode the green fluorescent protein（GFP）was constructed. Microprojectile bombardment was used to separately introduce two plasmids pJPM5 and pSBG700 into rice TNG67 calli. The transgenic plants were confirmed by Southern blot. Most of the transgenic plants contain 1 to 8 copies of transgene. GFP gene expression was measured in the extracts from rice leaf of two-month-old transgenic plants. And the quantification of *gfp* expression is

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carried by the SLM－8000 Fluorescence Analyzer. Most of the transgenic plants have showed significant GFP signal. Even though the rice plant has auto-fluorescence, the expression of GFP is high enough to give out much higher fluorescence signal compared with auto-fluorescence of rice plants. The GFP was also observed in transformed calli under microscope. The transient expression of GFP helped to demonstrate that the bombardment shots of 900psi & 1350 psi make the particles carrying plasmids reach more cells than the bombardment shots of 2X 900psi or 2X 1350psi. Our results showed that this modified GFP gene can be used as a reporter gene for rice genetic transformation and even for other cereal crops such as wheat and corn etc. The result also professes that the MAR sequence can enhance the expression of GFP gene significantly (data was shown in another paper).

Key words: Green fluorescence protein (GFP), Reporter gene, Genetic transformation, Rice (*Oryza sativa*)

The green fluorescence protein (GFP) gene, originally from the jellyfish *Aequora victoria*, has distinct advantages over alternative visual marker genes (Cao *et al*, 1992). Recently, a modified GFP gene which encodes a new version of GFP was identified (Crameri *et al*, 1996), which may facilitate the quantification of GFP expression in transgenic plants (Nagatani *et al*, 1997; Reichel *et al*, 1996). Because the new GFP form coded by the modified GFP gene is more effective in fluorescence emission and smaller in protein size than wild jellyfish originated GFP. The modified GFP has higher excitation and emission wavelength. Thus it is more convenient for the observation and quantification of fluorescence signal of GFP by microscope or other equipment. Therefore in our work, a modified GFP gene is used.

Since the jelly fish GFP gene was isolated, it has been used widely in many research works. It has been used in plants (Sheen *et al*, 1995), microorganism, animals etc for molecular biology research. In molecular approaches, GFP gene has been used mainly for the following research purposes (Cramari *et al*, 1996): A. Protein tagging--through fluorescence signal to monitor the location of a protein in living cells. If a gene of interest protein is fused with GFP gene, after transformation, the location of the protein can be monitored in living cells by observing fluorescence signal. Also by this method the movement of a protein among organs of a cell or even between cells can be detected. B. Developmental screen--GFP can be used to label gene, or even tissue in development tagging. For example, if GFP gene was introduced into tumor forming cells, then the cells were injected into nude mice. The tumor cells may develops in the nude mice, or even the tumor cells might transfer from one organ to another organ in the body. By observing the fluorescence *in vivo*, it can trace the development of tumor (cancer) inside the animal body. C. As a reporter gene, *gfp* has distinct advantages over alternative visual marker genes. As we know, conventionally in order to analyze expression of gene, such as GUS gene, NPT gene, we must add substrate to the tissue to be analyzed or extract the enzymes encoded by the genes from the tissues or transformants. These treatments will be damaged to the tissues or transformants. However, if *gfp* is used in genetic transformation in order to see whether *gfp* express or not, the expression of gene can be observed directly in living cells without damaging to the tissues or transformants .

In terms of the usage of *gfp* as a reporter gene, there are some reports which demonstrate the possibility of GFP to be used as reporter gene in plants. Several reports showed use of the GFP gene after introducing it into dicotyledonous plants (Sheen *et al*, 1995). Reports on using GFP gene in monocotyledonous plants are relatively few. One report showed that it can facilitate the selection of transformants in transformation. In that experiment, lower selection agent antibiotic was used in selection and regeneration culture. There are two reports mainly showing GFP expression pattern in transgenic plants. But the expression patterns of GFP gene are controversial in these two reports. So far reports showed GFP gene was introduced into lettuce, tobacco, apple and other plants. From these reports, it can be concluded that *gfp* has many advantages if it is used as a reporter gene and also it has this potentiality. Still more work can be done to further develop the applications of GFP in plant especially in cereal plants in term of molecular biology research. For *gfp* can be used as a reporter gene, it is necessary that the expression of *gfp* can be visibly observed first. Secondly, in terms of the application of GFP as a reporter gene in cereal crops, the possibility of quantification analysis of GFP is an important aspect.

Rice is a model plant for plant molecular biology research of cereal crops. Previous reports showed that cereal crops including rice, wheat, corn, have auto-fluorescence more or less especially in leaves. This kind of auto-fluorescence is caused by the exist of chlorophyll and other segments. Although in plant roots, the auto-fluorescence is less than in leaves, for *in vivo* observation and selection without damage to the whole transgenic plant, it is convenient to observe the expression of transgene at the leaves instead at roots. Therefore whether GFP gene can be used as a reporter gene in rice genetic transformation needs to be further investigated. Another reason we want to demonstrate the analysis results of *gfp* in transgenic rice is that the results or phenomenon in rice transformation may represent the possible results of other cereal crops such as corn, wheat, rye etc. Because cereal crops have many similar characteristics in terms of their physiological and structural traits.

1　Materials and Methods.

1.1　Construction of plasmids containing GFP gene in pJPM5

We used the pGHNC5 plasmid, reported by Allen *et al*. (1993, 1996), for the construction of pJPM5 (Figure 1). Plasmid pJPM0 was constructed by inserting a Klenow enzyme-filled *Eco*RI fragment, isolated from pBY505 (Wang and Wu, 1995; Zhao *et al*, 1989), which contains the *Pin*2 terminator, 35S promoter, *bar* gene and *Nos* terminator, into the *Sma*1 site of pGHNC5. Plasmid pJPM5 was constructed by inserting the rice *actin*1 promoter (McElroy *et al*, 1991) and a GFP gene isolated from pSBG700, into pJPM0. Plasmids pSBG700 was constructed by Jukon Kim and Ray Wu (unpublished results).

1.2　Production of transgenic rice plants

Calli were induced in MS medium (Murashige and Skong, 1962) from mature embryos of japonica rice (*Oryza sativa* L.) cv. TNG67. Fifteen-day-old embryogenic calli were bombarded with gold beads coated with either pJPM5, or with plasmid pSBG700 according to the procedure of Cao *et al*. (1992). Resistant calli were selected in plates containing MS medium (Wang and Wu, 1995; Zhao *et al*, 1989), supplemented with 6 mg L^{-1} Bialaphos as the selective agent, for 4 weeks (subcultured every 2 weeks). Resistant calli were transferred to plates containing MS regeneration media with 1 mg L^{-1} kinetin, 2 mg L^{-1} BAP, 0.25 mg L^{-1} NAA and 3 mg L^{-1} Bialaphos to regenerate into plants. Regenerated plants were transplanted into sterilized soil and grown in the greenhouse (30℃ day and 24℃ night with a supplemented photoperiod of 10 h).

The presence of the transgenes in regenerated plants was first inferred by the herbicide resistance of the plants. To test for herbicide resistance, one leaf from each of the one-month-old transgenic rice plants was painted on both sides with 0.25% (v/v) of the herbicide Basta (containing 162 g L^{-1} glufosinate ammonium: Hoechst-Roussel Agri-Vet Co., Somerville, NJ) and 0.05% (v/v) Tween 20. One week later, the resistant or sensitive phenotypes were scored.

1.3 Quantitative assay of GFP values in transgenic rice plants

To detect GFP value in R0 transgenic rice plants, a quantitative assay was carried out. The second leaf from the top of each two-month-old transgenic rice plant was collected and immediately frozen in liquid nitrogen. The leaves were ground in liquid nitrogen and homogenized in ice - cold extraction buffer (100 mmol HEPES, pH 8.0, 10 mmol EDTA, 5 mmol DTT, 10% glycerol, 1% PVP, 25 μg mL^{-1} PMSF, 15 μg mL^{-1} leupeptin, 1% activated carbon). This extraction buffer was found by preliminary experiments to give minimum autofluorescence of rice leaf extracts (data not shown). After the first centrifugation (12 000 rpm for 15 min at 4℃), the crude extract was centrifuged again (12 000 rpm for 20 min at 4℃) to further reduce insoluble particles which affect the quantification of fluorescence. Then the GFP values were quantified based on fluorescence units μg^{-1} protein using a fluorometer (model SLM8000) after excitation at 385 nm and measure the emission maximum at 510 nm. Non-transformed tissues were used to estimate the autofluorescence of the tissue. Protein concentration was determined according to Bradford (Bradford, 1976). Statistical analysis was carried out as described by Sokal and Rohlf (Sokal and Rohlf, 1969).

1.4 Transient assays of GFP values

Callus induction and particle bombardment were carried out as described by Cao *et al*. (1992). Fifty calli were used in each plate for each bombardment. Six replicate bombardments, totally 300 calli, were used for each plasmid. The amount of GFP in calli was roughly estimated by using a BH - 2 Olympus Fluorescence Microscope after 24, 36, 48, 60 and 72 hours of bombardment. The quantitative assay of transient GFP expression is carried according to the above described method.

1.5 DNA-blot hybridization analysis of transgenic rice plants

Genomic DNA from transgenic rice plants was prepared as described by Zhao *et al*. (1989). Eight micrograms of genomic DNA were digested with restriction enzymes, the DNA fragments were separated by electrophoresis through 1% agarose gels and transferred to nylon membranes (Nytran, Schleicher & Schuell). Probe preparation, hybridization and detection were performed using the α^{32}P-dCTP radioactive labeling system following the manufacturer ' s protocol (Gibco BRL).

1.6 Effect of bombardment He pressure on the number and position of cells bombarded.

In our experiment, we bombarded calli with three kinds of combinations of two shots for each plate containing calli: 900psi&900psi; 900psi&1350psi; 1350psi&1350psi. This experiment is to show the effect of different combination of two shots on the cell number bombarded and cell position bombarded inside the rice calli cluster.

2 Results

2.1 Transformation efficiency

The plasmids used in this study are depicted in Figure 1. Either pJPM5 or pSBG700 were used with a mixture of gold beads to bombard 300 - 350 calli. Transformation efficiency was calculated based on the number of independent transgenic plant lines regenerated, divided by the total number of calli used for particle bombardment. We found that between 15 and 20 plants were regenerated per 300 calli, and the efficiency was similar for both plasmids (data not shown).

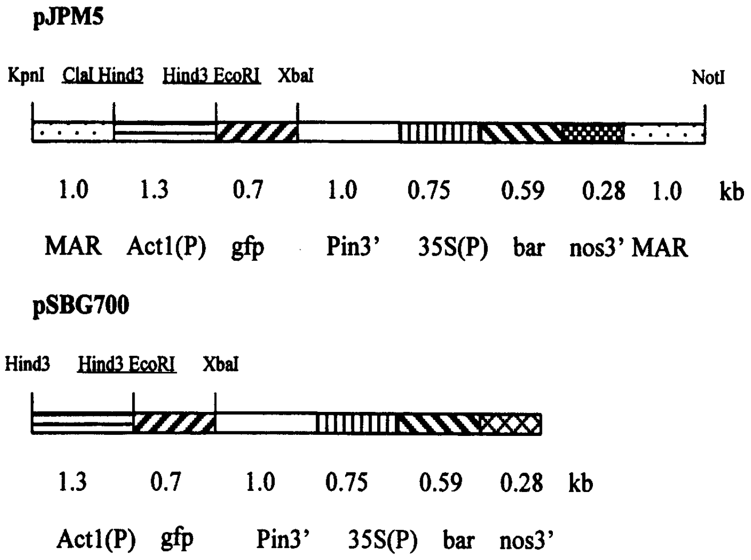
pJPM5
KpnI
ClaI Hind3
Hind3 EcoRI
XbaI
NotI
1.0 1.3 0.7 1.0 0.75 0.59 0.28 1.0 kb
MAR Act1(P) gfp Pin3' 35S(P) bar nos3' MAR
pSBG700
Hind3
Hind3 EcoRI
XbaI
1.3 0.7 1.0 0.75 0.59 0.28 kb
Act1(P) gfp Pin3' 35S(P) bar nos3'

Fig. 1 Schematic diagram of plasmids with gfp gene.

Act1 (p): rice *actin*1 promoter with *actin*1 first intron; *gfp*: a modified gene coding a green fluorescence protein; *bar*: the bacterial phosphinothricin acetyltransferase gene; Pin3': 3' terminator region of potato proteinase inhibitor 2 gene; 35S (p): cauliflower mosaic virus 35S promoter; nos: terminator region of the NOS gene;

2.2 Effect of bombardment He pressure on the number and position of cells bombarded.

Effect of bombardment He pressure on the number and position of cells bombarded is investigated through observing the transient expression of GFP in transformed calli. The transformation method is basically the same as described by Cao *et al*. (1992). The bombardment He pressure is determined by an experiment which is designed to get suitable He pressure. Usually two shots are used for each plate containing calli to be bombarded. Some people like to use two shots of 900psi, some like to use two shots of 1350psi. In previous work of other people, they used GUS as reporter gene (Jefferson *et al*, 1987). In those cases, it was not possible to reveal the real number of the cells bombarded as well as the cell layers that particles reach. Because the blue substrate produced by the GUS enzyme from X-Gluc may diffuse from bombarded cells to un-bombarded cells in the calli. Therefore it seems that 2 shots of 900 psi, 2 shots of 1350 psi or one 900 psi shot plus one 1350 psi shot have no difference in terms of the number and the layers of cells bombarded. However in our experiment, different plasmids containing GFP gene were introduced into 15 days old calli or 35 days old calli separately. The GFP is a protein which usually does not diffuse among cells. The location of bombarded cells from a layer to another layer can be detected through observing GFP by slightly changing the focus of the microscope. It is found through observing the GFP under microscope (Figure 4), that 2 shots of 900 psi mainly help particle into the top and second layer cells of calli. Two shots of 1350 psi help particle into the third and forth layer cells of calli. Besides, they have less cells bombarded. Because in some cells there were more than one particle if 2 shots of 900 psi or 2 shots of 1350 psi were used. However, one shot of 900 psi plus one shot of 1350 psi help the particle reach into the cells of top, second and third and forth layers of calli. We found that there are more cells bombarded by one shot of 900 psi plus one shot of 1350 psi than by only 2 shots of 900 psi or 1350 psi (diagramed in Figure 2). This result suggest that for rice genetic transformation by particle bombardment, it is better to use two shots of 900 psi plus 1350 psi to bombard the calli.

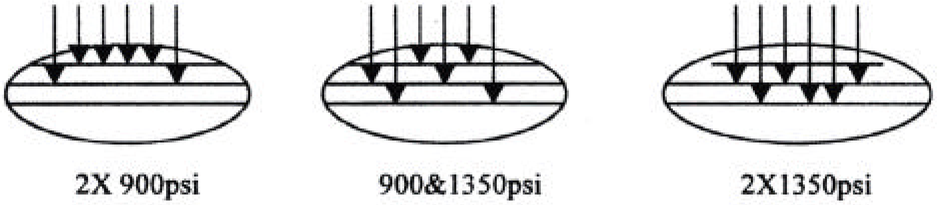

Fig. 2 The simplified schematic of the particle distribution in cells among different layers of the calli.

2.3 The quantification of *gfp* transient expression level

Non-bombarded calli were used as an internal control to set reference while quantifying GFP expression of the bombarded calli. GFP expression level was roughly estimated under a BH-2 Olympus Fluorescence Microscope (data not shown). It was found that *gfp* transient expression value was relatively high 36 hours or 48 hours after particle bombardment. Fifty random chosen calli were used in the quantitative assays of GFP transient expression. Therefore total proteins of transformed calli as well as non-bombarded calli were extracted 36 hours or 48 hours later after bombardment. GFP fluorescence units were measured at 490 nm of excitation wavelength and 510 nm of emission wavelength. The average relative GFP value in the pSBG700 control plasmid was 364 FLU μg^{-1} protein, whereas the values for pJPM5 was 620 FLU μg^{-1} protein, respectively (Table 2). Thus, from these experiments, the tobacco Rb7 MAR sequence increased the GFP value by 70%.

Table 1 Transient expression in calli bombarded with different plasmids. GFP level is shown as fluorescence units μg^{-1} protein.

Plasmid	Number of Calli Used in Assay	GFP Value	Percentage of Control
pSBG700	50	364	100
pJPM5	50	620	170

Each GFP value is the average of 3 samples. Unbombarded callus cells were used as control to set reference when GFP value was quantified in SLM800. The method is the same as the quantification of stable expression of GFP in transgenic rice plants.

2.4 DNA blot analysis of R0 transgenic plants

DNA blot experiments were carried out after digestion of the genomic DNA isolated from R0 transgenic plants that were each transformed with plasmids pJPM5 and pSBG700. DNA blots were first probed with *gfp* to show the presence of the transgene (data not shown). In order to roughly estimate the copy number of transgene, the filters were washed and then re-probed with the rice *actin*1 first intron (0.5 kb) (Cao *et al*, 1992). The result of DNA blot analysis is shown in Figure3. From the previous work of McElroy *et al*. (1991), it was found that there is only one endogenous copy of the 5' intron-containing *actin*1 gene in rice genome. There is also one copy of the 5' *actin*1 intron in pJPM5 or pSBG700. Therefore, by digesting the rice genomic DNA with an enzyme that cuts once in the plasmid (e. g., ClaI for Figure 3A, NotI for Figure 3B), and by using the 5' intron of *actin*1 as probe, the ratio of intensity of the hybridizing band that corresponds to the endogenous copy of the 5' intron of *actin*1 to the intensity of other bands can, in principle, give the copy number of the transgene. However, the exact copy number cannot be determined in these experiments. This is mainly because several high molecular weight DNA bands were too close to the band that contains the endogenous copy of the *actin*1 intron for clear separation. For example, in Figure 3A, lane 2, the 12 kb band appeared to contain two DNA fragments of slightly different sizes which were not separated, whereas in lane 6 the 12 kb endogenous band was partly separated from the adjacent band. Our gel electrophoresis conditions did not allow adequate separation of a 12 kb DNA from an 11 kb or 13 kb DNA fragment. Since pJPM5 was over 10 kb in size, and a rare-cutting enzyme was used to digest rice genomic DNA, we expect the *actin*1 intron containing DNA fragment to be large. The other problem is that the amount of DNA loaded into each lane was not strictly controlled.

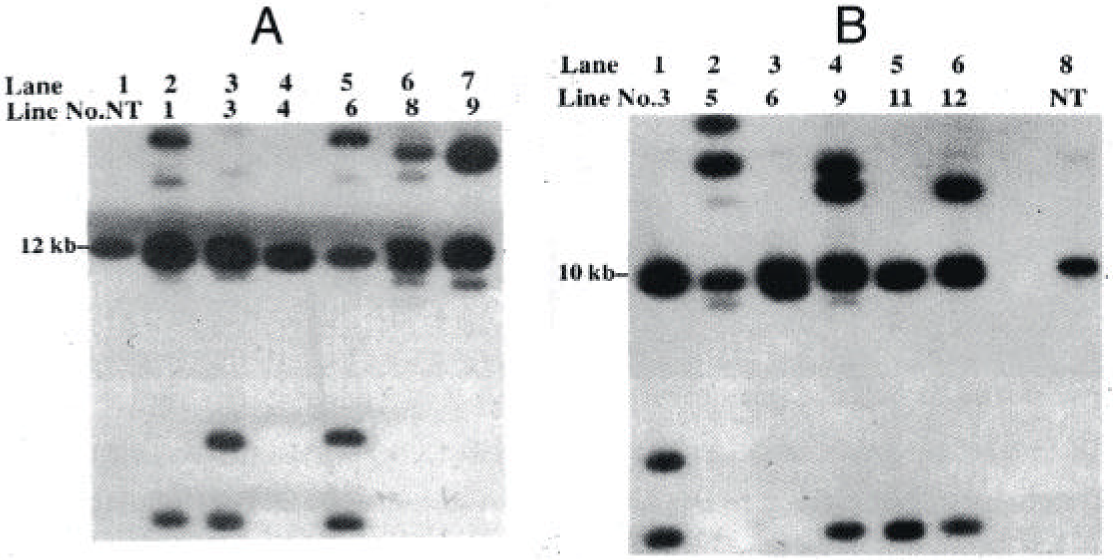

Fig. 3 DNA blot analysis of R0 transgenic plants.

DNA blot (Southern blot) analysis was performed as described in Materials and Methods. An α^{32}P-dCTP-labeled 0.46 – kb *act*1 intron DNA fragment was used as the probe. NT, nontransgenic plants. Copy number of transgene was determined as described by Duan *et al*. (Duan, 1996) using an internal standard: the first intron of *act*1, which exists as a single-copy sequence in the rice genome.

A. DNA-blot analysis of pJPM5 R0 plants. Genomic DNA (8 μg) from R0 transgenic rice leaf tissue was digested with *Cla*1 and loaded in each of lanes 2, 3, 4, 5, 6 and 7. DNA from NT was digested with *Cla*1 and loaded in lane 1.

B. DBA-blot analysis of pSBG700 R0 plants. Genomic DNA (8 μg) from R0 transgenic plant leaf tissue was digested with *Not*1 and loaded in each of lanes 1, 2, 3, 4, 5 and 6. DNA from NT was digested with *Not*1 and loaded in lane 8.

Thus, we cannot compare the DNA band density between different lanes, including those representing non-transgenic control plants (NT). In spite of these problems, we can draw the conclusion that most transgenic plant lines contain between 1 and 8 copies of the transgene. For example, the hemizygous R0 plant line No. 6 in Figure 3A seems to contain three copies of the transgene (approximate sizes > 15 kb, 4 kb, and 1 kb, since the density of each band is half that of the 12 kb band, which comes from the endogenous copy of the *actin*1 intron, which is homozygous). In plant line No. 6, the two low molecular weight bands (approximately 4 kb and 1 kb) most likely represent rearranged input plasmid.

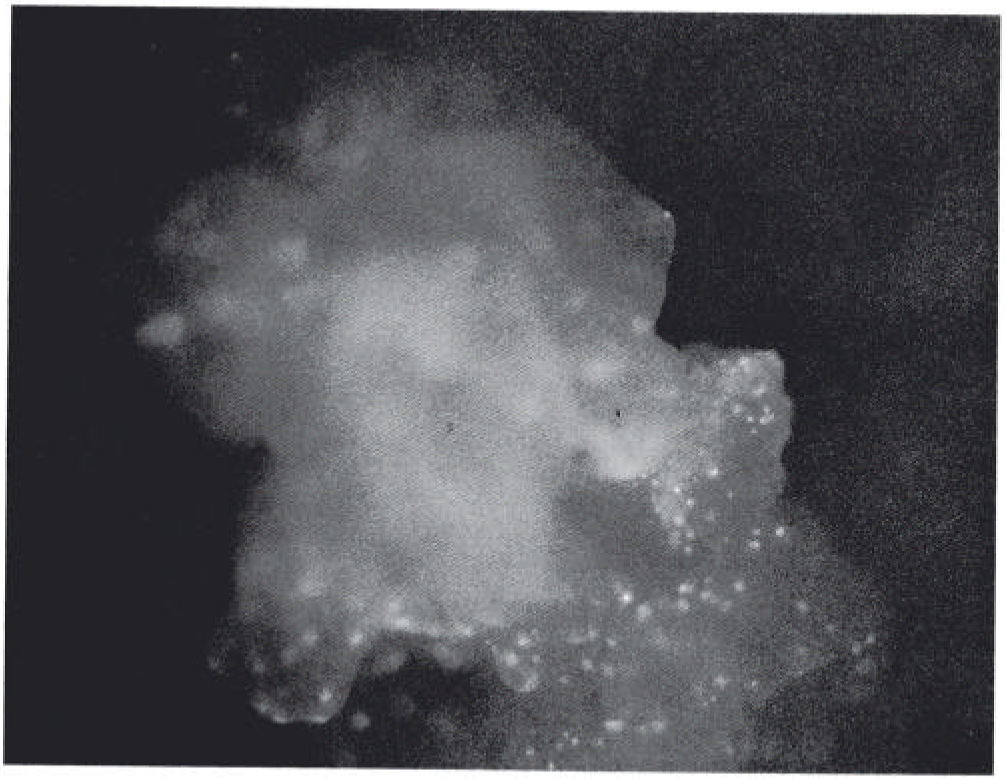

Fig. 4 Transient expression of GFP gene in bombarded calli 2 days after bombardment.
The bright white spots indicated with arrowhead are caused by the fluorescence signal under a BH-Olympus Fluorescence Microscope

2.5 Quantitative analysis of GFP gene stable expression in transgenic plants.

For quantitative analysis of GFP, it is important to avoid degrade of protein. Secondly to eliminate the auto fluorescence of extract from green parts of plant due to the exist of chlorophyll and other segments. So we add some activated carbon to reduce the effect of chlorophyll and other segments. From color observation, if activated carbon is added in the extract solution, then the extracts of rough protein is clear, otherwise, it is not clear with the green-yellowish color. Adding suitable proteinase inhibitor can contribute to stabilization of protein while measuring the fluorescence in the machine. Since during measurement, protein extract solution can not always be kept at low temperature 4℃. The solution temperature may rise gradually to room temperature. If there is degrade of proteins including GFP, then the value of fluorescence may gradually decline, which cause the variation of fluorescence of the measurement. In non-transformed plants, the autofluorescence value averaged 5.8 FLU

μg^{-1} protein (Table 1, top half). GFP value in 12 R0 generation transgenic plants (pSBG700) ranged from 16.8 to 84.1, with an average of 47.1 FLU μg^{-1}. GFP value in 10 R0 plants (pJPM5) ranged from 605 to 1333, with an average of 867 FLU μg^{-1} protein, an average which is 18 – fold higher than those plants harboring pSBG700. From this experiment, we found that MAR sequences significantly enhanced *gfp* stable expression (data was shown in another paper).

Table 2　The levels of green fluorescence (FLU μg^{-1} protein, top half) in transgenic and non-transformed rice plants.

Plasmid	Number of R0 Lines	Range of GFP Value	Average GFP Value or (fold difference)[a]	Variation in Transgene Transgene Expression
pSBG700	12	16.8 – 84.1	47.1[b] (1.0)	5.0 fold
pJPM5	10	605 – 1333	867[c] (18.4)	2.2 fold
None	6	4.4 – 8.0	5.8	

a. Fold difference is the difference between plasmids with the MAR sequence as compared to those (pSBG700 with the value set at 1.0 fold) without the MAR sequence. The values were not corrected for the background in non-transformed control. b. Standard error is 3.2; standard deviation is 12.5. c. Standard error is 65.8; standard deviation is 132.2.

3 Discussion

3.1 GFP gene is very useful to determine gene expression in cells or organs exactly.

Because GFP is a protein, it will not diffuse among organ or cells. Thus the GFP will always exist in the cells or organs where it is expressed. The expression position might be in chloroplast, mitochondrion, cell membranes or even nucleus.

If GUS gene is used as a reporter gene, it is difficult to locate the exact position of gene expression (Iglesias *et al*, 1997; McElroy *et al*, 1991). Because the determination of GUS gene expression is based on the mechanism: GUS enzyme to degrade X-Gluc, a foreign chemical added, and the sub chemical would show blue color. Both X-Gluc and its degraded sub chemical are small moleculars compared with protein. They can diffuse among organs, membranes, cells, or even among plant tissues. For example, if GUS expression happened in chloroplast, even though the GUS enzyme only exist in chloroplast, the blue sub chemical of X-Gluc would diffuse from chloroplast to the whole cell or even diffuse to other cells. This phenomenon makes it difficult to locate the requisite position of transgenic expression. In term of this point, GFP gene is better than GUS gene as a reporter gene.

3.2 It is possible to quantify GFP expression by fluorescence measurement even though there is auto fluorescence somewhat in green parts of plant Some plants have auto fluorescence due to the exist of chlorophyll and other segments. The ability of auto-fluorescence is high or low, whether it will affect the observation of GFP is a major concern issue for the application of *gfp* as a reporter gene. Our result show that GFP has significant higher fluorescence signal compared with auto-fluorescence signal.

3.3 The results show that GFP is also an ideal reporter gene for the research of genetic transformation. In some aspects, it is better than GUS gene as reporter gene.

GUS gene is a conventional reporter gene. It is used as an important tool in the research work in plant and microorganism molecular biology. Even though it is a good reporter gene in some aspects, it has disadvantages. First of all, for the determination of GUS gene expression, plant material should be cut off and treated with 4 – MU or X-Gluc. X-Gluc is a tumor-inducing chemical. Therefore it needs to be handled very carefully. Plant cannot be determined of GUS gene *in vivo*. In contrast the GFP can be visualized directly observed under microscope *in vivo* without the addition of exogenous substrates or cofactors, and it is not toxic. Thus GFP gene is an ideal reporter gene for the vitro selection of transformants.

3.4 Different expression patterns of GFP gene in transient expression of transformed calli and stable expression of transgenic plants.

From the result shown in Table 1, for the pSBG-transformed transformants, stable expression of GFP in transgenic plants has much lower fluorescence units than in transient expression in transformed calli. This is caused by the fact that in transformed calli, 36 – 48 hours after bombardment, every transformed cell may hold several plasmids (more specifically *gfp*). However, some plasmids may got lost while the transformed cells regenerated into plants. That means some plasmids did not integrate into rice chromosome. For the pJPM5-transformed calli, the transient expression level of *gfp* is lower than in stable expression of transgenic plants. This is due to the effect of MAR sequences (this is demonstrated in another paper).

In general speaking, by observing fluorescence under microscope or measurement of the fluorescence signal in the spectrometer, GFP expression can be confirmed or quantified. Therefore GFP gene is a sensitive and vital reporter gene for rice genetic transformation.

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